Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF COPPER STRESS ON BIOCHEMICAL PROFILING IN *DUNALIELLA SALINA* (TEOD.) AND *SPIRULINA PLATENSIS* (GOMO.)

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ABSTRACT

Growth, lipids, carbohydrates as well as fatty acid compositions of a native microalga in *Dunaliella salina* (Teod.) and *Spirulina platensis* (Gomo.) were studied in batch culture at light intensity 100 µ mol photons m^{-2} s^{-1} temperature 25 ± 1°C and 16:8 h light and dark diurnal cycles. Carbohydrate content decreased with increasing concentration of Cu stress. The lipid content also showed the same scenario. Further GC-MS profiling of lipids showed various compounds separated according to their retention time in which most of the compounds were fatty acid methyl ester. With further augmentations of lipid & carbohydrate content and improved fatty acids, the native microalga strain could be a potent candidate for aqua-culture feeding and or biofuel production.

KEYWORDS: ***Dunaliella salina, Spirulina platensis, lipids, carbohydrates, GC-MS profiling, Cu stress***

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Received on : 05-10-2016
Revised and Accepted on : 17-01-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.p235-244

INTRODUCTION

Microalgae biomass contains products with high commercial importance like proteins, lipids, carbohydrates [1] Nutrient availability has a significant impact on growth and propagation of microalgae and broad effects on their lipid and fatty acid composition. Environmental stress condition when nutrients are limited invariably causes a steadily declining cell division rate. Surprisingly, active biosynthesis of fatty acids is maintained in some algae species under such conditions, provided there is enough light and CO_2 available for photosynthesis [2] *Spirulina* is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. Their main photosynthetic pigment is phycocyanin, which is blue in colour. These bacteria also contain chlorophyll a and carotenoids. Some contain the pigment phycoerythrin, giving the bacteria a red or pink colour. *Dunaliella* species belong to the phylum Chlorophyta, order Volvocales and family Polyblepharidaceae, and are unicellular, photosynthetic and motile biflagellate microalgae morphologically distinguished by the lack of a rigid cell wall[3] one who has grown microalgae under laboratory or outdoor condition is well aware of the fact that to obtain high lipid content, external stress or lipid induction techniques need to be applied. Many microalgae produce saturated and unsaturated fatty acids naturally under ideal growth conditions, which have high nutritional value, but are less ideal for biofuels. However, the synthesis of neutral lipids in the form of Triacylglycerides (TAGs) can be induced in many species under stress conditions[4,5]. There has been a wide range of studies carried out on lipid induction techniques in microalgae such as the use of nutrients stress, including nitrogen and/or phosphorus starvation [6] Lipids are secondary metabolites produced by microalgae helps in maintaining specific membrane functions and cell signaling pathways while responding to the environment changes. The fatty acid content is influenced by the environmental and cultural conditions of microalgae [7] Algal lipids have been recognized as suitable feedstock for biodiesel production [8] Algae synthesize fatty acids principally for esterification into glycerol based polar lipids under favorable conditions. It is well known that some heavy metals which are essential component in metabolism are toxic when present in high concentration [9] carbohydrates are the copious class of organic compound and are foremost source of energy. Carbohydrates are initially synthesized in plants from a complex series of reactions involving photosynthesis. These carbohydrates are not only the storehouse of energy but they also serve as a structural material (cellulose), a component of the energy transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA. Different methods for determining reducing sugars, sucrose and starch have been described by many workers [10,11]. Since heavy metals exert toxic effects on living organisms, they are termed toxic heavy metals. Some of the heavy metals, such as copper, nickel, and zinc, are, at very low concentrations, essential for life (also termed microelements or trace elements) because they play important roles in metabolic processes taking place in living cells [12] However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Copper and zinc are essential heavy metals, biologically active, play a role in photosynthesis and as catalytic agents in some enzymatic reactions, but when in excess, they are extremely toxic to cells. Heavy metals like these are essential micronutrient required for a wide variety of cellular processes. In the present study, two algae *Dunaliella salina* (Teod.) and *Spirulina platensis* (Gomo.) were subjected to different concentrations of copper stress and the effect on the carbohydrates and fatty acid profiling was investigated. The aim of this study is to determine if these biochemical parameters can be used as sensitive physiological parameter in toxicological studies of heavy metals on these algae.

MATERIALS AND METHODS

Isolation, purification, Identification and Cultivation of algal strains from different areas of Jaipur.

Experimental Methods

Periodic collections of algal samples (*Spirullina platensis)* were collected at different sites of Jaipur city especially from Jal Mahal and Tal Katora and other algae (*Dunaliella salina)* was collected from Sambhar Lake (Rajasthan). (Table1) From different water bodies cultures of different algal strains were obtained and were purified and maintained in suitable culture medium. For their optimum growth, cultures were maintained in agar slants and cultured in liquid medium. The cultures were subculture once in every 25 days and cultures of slant were sub cultured once in three months.

Isolation and Purification

By centrifugation method algal samples with very low cell count and in mixed forms were concentrated, while those with high cell counts were diluted with suitable medium. Enrichment and isolations were carried out using enrichment culture media till unialgal forms of species were obtained. While doing simple enrichment method, the inoculum was prepared by mixing the samples with the selected medium and then serial dilutions were made in test tubes containing similar media. Direct isolations were done by picking up single filament or single cell using sterile Pasture pipettes, which were pulled out to a very thin capillary, using a dissecting microscope. Streaking method was used to contaminated cultures which can be made unialgal. In streaking method, a loop full algal suspension is taken and drawn into a long zigzag streak on an algal plate. After incubation, isolated colonies appearing at the tail end of the streak are expected to be unialgal.

Maintenance of Stock Cultures

The unialgal cultures were grown on agar slant with suitable medium contained in screw capped culture tubes to reduce frequent sub culturing without losing viability. The inoculated slants in 10-15 ml culture tubes with cotton plugs were incubated in a growth room for 7-10 days. The cotton plugs were replaced by pre sterilized, bakelite screw caps provided with rubber liner under aseptic conditions. After sufficient algal growth appears on the agar slants, culture tubes were then transferred to stock culture room under conditions which

were just sufficient to keep them viable state. Low temperature (15-20°C) and light intensity (50-200 lux) maintains the culture in viable state for longer duration.

Cultivation

For the cultivation 3-5 day old cultures of algae of prepared inoculum of unialgal culture was added to 250 ml conical flasks containing 100 ml culture medium and subjected to controlled culture conditions. The cultures were grown with photoperiod of 12 hours light/dark provided by white lamps at a light intensity of 2,500 lux and temperature of 26 ± 2 °C. The cultures were shaken gently twice a day, as shaken cultures in contrast to the static ones have demonstrate better growth.

Bioaccumulation of Inorganic medium by Spirulina platensis and Dunaliella salina from growth medium with diverse microelement content.

Growth media

The composition of the growth medium in the case of *Spirulina platensis* was in accordance with Zarrouk's medium while for *Dunaliella salina* the OECD 201, AAP conditions were applied. Cu (II) metal at different concentration (0.5ppm, 2ppm, 4ppm and 6 ppm) was chosen for experiments. Five different solutions were prepared in case of each microelement: one control solution and 2 solutions with diverse metal concentrations [13] Alga suspensions with 10 mgl^{-1} concentration were prepared in Erlenmeyer-flasks (500 ml) by the application of the above described growing media. The experiments were performed in triplicates (n=3) and after the incubation time the whole amount of the gained alga was analyzed. The growing period was 2 weeks; the temperature was 25°C in case of *Dunella* and 32°C in case of *Spirulina.*

Biochemical Profiling

Total Soluble Sugars

The algal material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method of [11] Distilled water was added to make up the volume up to 50 mL and processed further for quantitative analysis.

Starch

Extraction

The pellet was dissolved in 5 mL of 52% perchloric acid [10]. 6.5 ml of distill water was added to raise he volume and was shaken.

Quantitative Estimation

Glucose was used as standard. To 1 mL of sample of both TSS and starch phenol and sulphuric acid was added.[14] The optical density was measured at 490 nm using spectrophotometer (Carl Zeiss, Jena DDR, VSU 2 P), after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Lambert Beer's Law. All samples were analyzed in the same way as described above and contents of total soluble sugars and starch were calculated by computing optical density of each o the samples with standard curve.

Lipids

Extraction and Quantification

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v;) [15] . The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

GC-MS Profiling

Gas Chromatography and Mass Spectroscopy (GC-MS) The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280°C at 1 to 40 °C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 mL/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

RESULTS AND DISCUSSION

The screening of algal strains was carried out to select the potential algal strains in terms of biomass and bio pigment production especially carotenoids and phycocyanin. Blue green algae is screened as a potential source of biomass and biopigments so strains were compared for their biomass and biopigment i.e. chlorophyll – a, carotenoids, phycocyanin, allophycocyanin and Phycoerythrin composition of the cyanobacteria. Growth (i.e. optical density and dry weight) and productivity (growth rate) analysis of different algal isolates showed significant difference in growth pattern. On the 25th day of experiment maximum growth i.e .optical density and biomass (as dry weight) respectively was observed in cultures of both the algae. A screening of the blue green algal strains was carried out to select the elite strains in terms of biomass and pigment levels which could be a potential source of biopigments. It has reported [16] various inorganic salts as precursors in media for optimum growth in *Dunaliella salina.* On going through results of biomass production,

growth rate and economically important pigments i.e. phycobiliprotein, Jaipur isolated strain *Spirulina platensis* were found to be best for pigment biosynthesis except carotenoid. The first step prior to mass culture of algae in open ponds or bioreactors is to select the strains most suitable for mass culturing in terms of biomass, rate of growth and products of commercial value. Growth is a good indicator to determine the effect of any toxic compound in susceptible microorganism since it reflects the metabolism of the cell. Therefore, carbohydrates and lipids were observed in order to estimate the growth of both the algae. It was observed that in Cu stress (0.5 to 6ppm) *Spirulina platensis* maximum amount of carbohydrates was observed at 0.5 ppm (3.7 mg/gdw) while minimum was at 6 ppm dose level (0.88 mg/gdw) while in *Dunaliella salina* it was observed that maximum carbohydrates content was at 0.5 ppm (3.15mg/gdw) while minimum at 6 ppm dose level (0.54 ppm). Overall *S. platensis* had better carbohydrate content than *D. salina*. In case of lipids it was observed that when Cu stress was applied maximum content was observed at 0.5 ppm (10.64mg/gdw) and minimum at 6 ppm (4.86mg/gdw) in *S. platensis* and in case of *D. salina* maximum amount was at same dose level (10.32mg/gdw) and least in 6ppm (4.21mg/gdw). In the present investigation both carbohydrates and lipid content decreased with increase in concentration of stress (Table 2-5 -4 These findings are in agreement [17] who studied the biochemical changes in response to metal tolerance in *Anabena doliolum* exposed to Cu and Cd. The growth inhibition and decrease in biomolecules may be due to inhibition of photosynthesis, enzyme system, protein content, pigment degradation and functioning of PSII in both the algae. It has been reported by several workers [18] hat addition of heavy metals cause alteration in whole chain and electron transport activities. The reduction in content may be due to inhibition of normal cell division by binding at sulphydryl group. Further GC- MS profiling of control in *Spirulina* revealed many compounds which were mainly observed at retention time of 9.37, 13.43, 16.59, 19.89, 24.75, 33.17, 26.15 and 28.87 min. Similarly in Cu stressed culture of *Spirulina* major compounds were observed at 5.92, 8.05, 14.79, 17.71, 21.46, 25.26 and 29.62 min. respectively (Fig. 1 and 2 and Tables 6 and 7). Many compounds were observed in GC- MS profiling of *Dunaliella salina* (control). Majority of the compounds were observed at 7.32, 10.86 13.43 15.38, 17.94, 23.31 and 26.41min respectively while in Cu stress most of the compounds were observed at 5.12, 7.31, 9.13, 13.42, 17.93, 24.19, 27.20, 30.03 and 31.32 (Fig. 3 and 4 and Tables 8 and 9).

Figure 1
Peak representing retention time of various compounds identified by GC –MS in Spirulina platensis (Control)

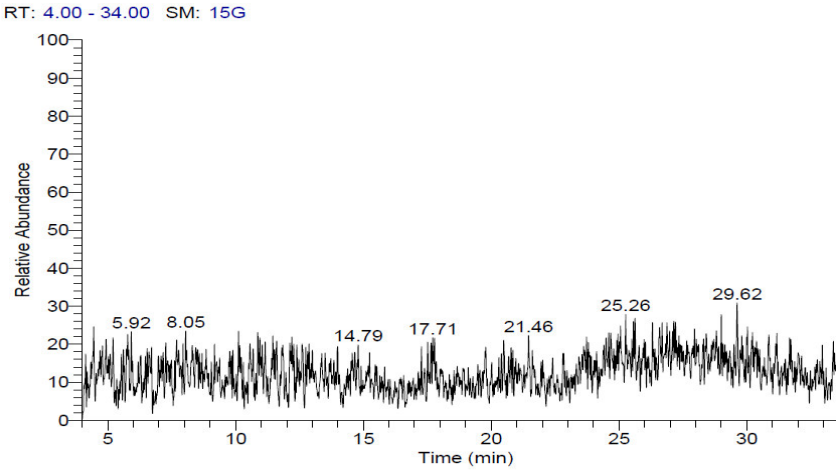

Figure 2
Peak representing retention time of various compounds identified by GC –MS in Spirulina platensis (With Cu stress at 4 ppm)

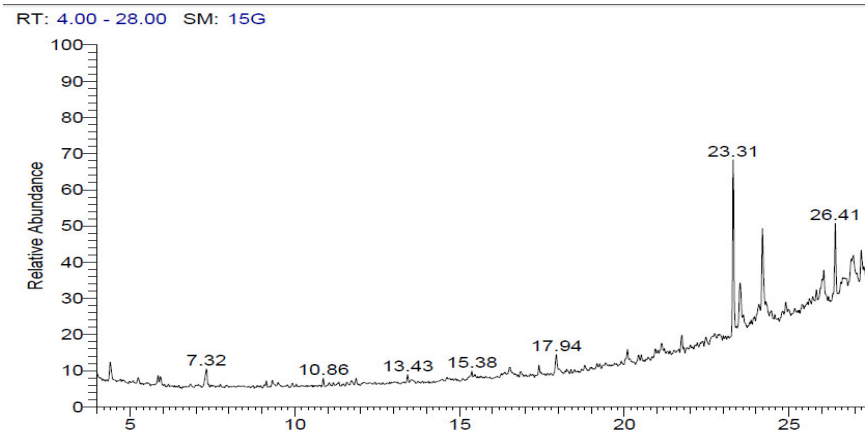

Figure 3
Peak representing retention time of various compounds identified by GC –MS in Dunaliella salina (Control)

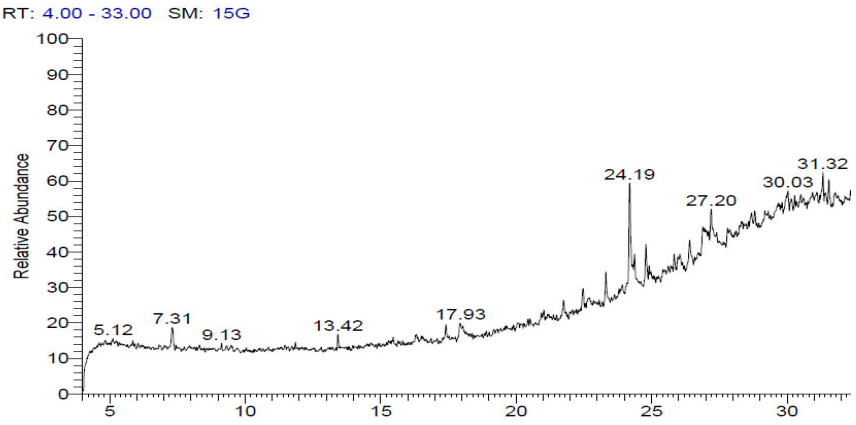

Figure 4
Peak representing retention time of various compounds identified by GC –MS in Dunaliella salina (with Cu stress 4 ppm)

Table 1
Culture Collection of algae

S.No.	COLLECTION SITE	MONTH	Name of algae collected
1.	JAL MAHAL,JAIPUR	AUGUST 2014	*Spirullina platensis*
2.	SAMBHAR LAKE, JAIPUR	NOVEMBER 2014	*Dunaliella salina*
3.	TAL KATORA,JAIPUR	JULY 2015	*Spirullina platensis*

Table 2
Effects of Copper stress on carbohydrate contents (in mg/gdw) on Spirulina platensis

CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
3.29±0.02	**3.7±0.04**	**2.80±0.009**	**1.31±0.007**	**0.88±0.003**

Table 3
Effect of Copper stress on carbohydrate contents (in mg/gdw) on Dunaliella salina

CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
3.11±0.01	**3.15±0.01**	**2.87±0.009**	**1.05±0.006**	**0.54±0.001**

Table 4
Effects of Copper stress on lipids contents (in mg/gdw) on Spirulina platensis

CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
10.21±0.83	**10.64±0.9**	**7.39±0.76**	**6.54±0.66**	**4.86±0.52**

Table 5
Effects of Copper stress on lipids contents (in mg/gdw) on Dunaliella salina

CONTROL	0.5 ppm	2 ppm	4 ppm	6 ppm
10.13±0.78	**10.32±0.87**	**6.45±0.62**	**5.89±0.57**	**4.21±0.44**

Table 6
Various compounds identified by GC –MS in Spirulina platensis (Control)

RT	Compound Name	Area	Area %
6.52	Methyl 4methyl4nitroso2trimethylsiloxypentanoate	16205	2.32
7.45	5[2(1,3Dioxolan2yl)ethyl]2methyl1cyclopentene1carboxaldehyde	11389	1.63
7.63	Malonic acid, 3methylpentyl propyl ester	10373	1.49
9.15	3 [2(4Methylphenylthio) ethyl]4Hsydnone	11358	1.63
9.37	1H1Pyrindine2,7dione, 1[2(1,3benzodioxol5yl) ethyl]3,4,5,6tetrahydro	26027	3.73
9.60	Benzene, (1nitropropyl)	12159	1.74
10.21	1,2Benzendiol, O,O'di(cyclopentanecarbonyl	12289	1.76

11.53	1,3Dioxolane, 2(phenylmethyl)	10203	1.46
12.32	2Methyl1dimethyl(dichloromethyl) silyloxypropane	8488	1.22
12.67	Phosphonic acid, (3methyl3penten1ynyl) diethyl ester, (E)	13708	1.96
12.81	Penicillamine, triTMS	9282	1.33
13.05	3Methylthio6phenyl5oxo4,5dihydro1,2,4triazine	13766	1.97
13.12	3Hepten1ol, acetate	18248	2.61
13.20	1Pentanone, 1(2pyridinyl	16406	2.35
13.31	Carbonic acid, butyl cyclohexyl ester	9136	1.31
13.43	NDifluorophosphoxyOtrimethylsilylhydroxylamine	44080	6.32
13.67	1Hexyl2nitrocyclohexane	14569	2.09
13.90	Butanamide, N(1oxopropyl)	17737	2.54
15.50	áDGlucopyranoside, methyl 2(acetylamino)2deoxy3,4,6triOmethyl	13751	1.97
15.57	Decane, 4methylene	8240	1.18
16.59	4Nonene, 1bromo, (Z)	11663	1.67
17.44	5Decene, 1bromo, (Z)	12119	1.74
17.53	Benzenamine, N[2methoxy1(methoxymethyl) 2methylpropylidene	15832	2.27
18.07	DarabinoHex1enitol, 1,5anhydro2deoxy	6205	0.89
18.12	2Cyclohexen1ol, benzoate	11922	1.71
18.24	Methyl 6anisoyládgalactopyranoside	11119	1.59
18.34	Benzeneacetamide, àethylNformylàhydroxy	14167	2.03
18.77	4Tridecanol	7911	1.13
19.39	Benzenepropanoic acid, ethyl ester	9336	1.34
19.89	Cyclobutanone, 2methyl2oxiranyl	10657	1.53
20.15	Tetradecanoic acid, 13oxo, methyl ester	18666	2.67
20.90	Phosphorochloridous dihydrazide, hexamethyl	15411	2.21
21.67	Propane, 3,3dichloro1,1,1,2,2pentafluoro	19227	2.76
22.13	Trimethyl (3,3difluoro2propenyl) silane	11528	1.65
22.26	Benzeneacetic acid, àmethyl3phenoxy, trimethylsilyl ester	18620	2.67
22.56	Silane, 2cyclohexen1yltrimethyl	9589	1.37
22.89	Propanedioic acid, propyl	16763	2.40
23.21	7Oxo4,7dihydrotriazolo(3,2c) triazine	20475	2.93
23.96	[1,4]Dioxino[2,3b] 1,4dioxin, hexahydro2,3,6,7tetramethyl	27552	3.95
24.74	àDXylofuranoside, methyl	14916	2.14
26.82	NBenzyloxy2isopropoxycarbonylazetidine	6149	0.88
31.95	Methyl 4methyl4nitroso2trimethylsiloxypentanoate	9886	1.42
33.17	Butanoic acid, 3methyl, trimethylsilyl ester	18015	2.58
33.29	N,O bis (trimethylsilyl)5(aminomethyl) isoxazol3ol	13166	1.89
33.51	3Hexene, 1(1ethoxyethoxy) (Z)	10548	1.51
34.05	Cyclohexanol, 2(trimethylsilyl), cis	10591	1.52
34.27	1Trimethylsilyloxynoctene	8803	1.26
35.28	Butanoic acid, 2methylcyclohexylester, cis	14408	2.06
35.52	(3Nitrobenzyl) phenethylamine	10482	1.50
35.93	[(Pyridine2carbonyl) amino] Acetic acid, ethyl ester	14679	2.10

Table 7

Various compounds identified by GC -MS in Spirulina platensis (with Cu stress at 4 ppm)

RT	Compound Name	Area	Area %
4.13	Ethanol, 2nitro, propionate (ester)	11447	1.85
4.44	tertButyl glycidyl ether	14335	2.32
5.20	Cyclopropane, 1butyl2(2methylpropyl	9227	1.49
5.54	Albuterol	8316	1.35
5.61	1,2Dithiepane	10145	1.64
5.78	Isobutyl (1((1methoxypropan2yl) oxy)propan2yl) carbonate	16538	2.68
5.92	Pyrrole, 1[(àmethylbenzylidene) amino	16237	2.63
6.54	Benzyl but2enoate	9570	1.55
6.71	1Nitroso3,5dinitrohexahydro1,3,5triazine	13284	2.15
7.62	Cyclohexanone, 2,2diethyl	7616	1.23
7.69	1Methyl1ethyl2[2(methoxycarbonyl)prop2yl] hydrazine	16400	2.66
7.94	N(4Oxo2thioxo1,4dihydro3(2H)quinazolinyl) 3pheny lpropanamide	8986	1.46
8.05	2Propenamide, 3methoxyNphenyl	10605	1.72
9.17	Trimethylsilyl 2,2,3,3,3pentafluoropropanoate	10105	1.64
9.89	Benzeneacetic acid, à(aminocarbonyl)àethyl	10262	1.66
10.11	5(Phenylsulphonyl)dihydro1,3,5dioxazine	13235	2.14

10.87	Iron, tricarbonyl(1,3,5,7cyclooctatetraene1carboxaldehyde)	23922	3.88
10.95	5Isoxazolecarboxylic acid, 4,5dihydro3phenyl	9543	1.55
11.16	1Phenylhexa1,5dione	8611	1.40
11.46	Phosphonic acid, (1amino2methylpropyl), bis(trimethylsilyl) ester	17419	2.82
11.52	Benzonitrile, mphenethyl	9906	1.60
11.61	Benzimidazol2(3H)thione, 1benzyl3morpholinomethyl	23647	3.83
12.12	3,4Hexanediol, 3,4dimethyl	10109	1.64
12.21	1(3Phenyl2prop2ynyl) 1,4dihydrobenzoic acid	11300	1.83
12.27	Silane, 2cyclohexen1yltrimethyl	11736	1.90
12.39	Cycloheptene, 5bromo	13018	2.11
12.60	Phosphoramidothioic acid, O,Odimethyl ester	22095	3.58
12.75	Cyclopropanecarboxylic acid, 2biphenyl ester	12055	1.95
12.87	Ethane, 1,1,2,2tetramethoxy	9176	1.49
17.27	Trimethyl(3,3difluoro2propenyl) silane	8903	1.44
17.51	2,5Dimethyl5hexen3ol	10758	1.74
17.78	4Hepten2one, (E)	9403	1.52
20.49	4Piperidinecarboxylic acid, 1nitroso	14157	2.29
23.80	Glutaric acid, cishex3enyl isohexyl ester	7471	1.21
25.26	2,2Diphenyl3methyl4morpholinobutyramide	12753	2.07
25.57	3Trimethylsilyloxytridecane	10833	1.75
25.63	Pentanoic acid, phenyl ester	13517	2.19
26.31	Silane, 2butenyltrifluoro	11084	1.80
26.69	1,3Bis(trimethylsilyl)but1ene	11441	1.85
27.14	2,6Piperidinedione, 4,4dimethyl	14390	2.33
27.21	3Methylpentan3yl propyl carbonate	9688	1.57
27.96	ádErythrohexopyranoside, methyl 2,3dideoxy4,6O(phenylmethylene	7716	1.25
29.00	Ethane, 1,1,2,2tetramethoxy	14801	2.40
29.62	Cycloheptane, 1,3dichloro, trans	33811	5.48
30.03	4Pentamethyldisilyloxyhexadecane	9559	1.55
30.23	N-MethoxyNtrifluoroacetyl1,1dimethyl2carbomethoxye Thylamine	8495	1.38
31.18	N-MethoxyNtrifluoroacetyl1,1dimethyl2carbomethoxye thylamine	8038	1.30
31.68	Benzenesulfonyl azide	8975	1.45
31.72	Pentanoic acid, 2hydroxy, ethyl ester	14168	2.30
32.95	Trimethyl(3,3difluoro2propenyl) silane	8458	1.37

Table 8
Various compounds identified by GC –MS in Dunaliella salina (Control)

RT	Compound Name	Area	Area %
4.40	Tetrakis (methylsulfonyl)hydrazine	3508655	1.99
5.85	N,N'Bis [(5Z) cyclooctenylidene] hydrazine	1140917	0.65
5.92	2Ethyl1hexanol, trifluoroacetate	1329230	0.75
7.32	3Methylheptyl acetate	3593413	2.03
9.14	Decane, 2,5,6trimethyl	598073	0.34
10.86	10Methylnonadecane	785126	0.44
13.43	Sulfurous acid, butyl decyl ester	866103	0.49
15.38	Oxalic acid, hexyl neopentyl ester	690735	0.39
16.54	4Hydroxyàbromoethylphenone	1164150	0.66
17.41	Oxalic acid, hexyl neopentyl ester	1603320	0.91
17.94	Phthalic acid, ethyl pentyl ester	3940032	2.23
20.10	Cyclohexanemethyl propanoate	3602613	2.04
20.44	2RAcetoxymethyl1,3,3trimethyl4t(3methyl2buten1yl) 1tcyclohexanol	1098297	0.62
20.52	2,2,7,7Tetramethyloctane	674794	0.38
20.94	DL4,5Octanediol	1123096	0.64
21.14	1Cyclohexylnonene	2136659	1.21
21.75	Dodecanal	3882763	2.20
22.38	1Hexyl2nitrocyclohexane	1811283	1.03
22.48	1Hexyl2nitrocyclohexane	1743061	0.99
22.75	2Hexyl1octanol	3970883	2.25
22.84	2Hexyl1octanol	2627666	1.49

23.31	Pentadecanoic acid, 14methyl, methyl ester	25890686	14.65
23.52	2Hexyl1octanol	10534030	5.96
23.62	DL4,5Octanediol	2439309	1.38
23.92	1Hexyl2nitrocyclohexane	378959	0.21
24.09	1Octene, 3,3,4,4,7,7,8,8,8nonafluoro	4399269	2.49
24.20	Oxalic acid, allyl octadecyl ester	19736681	11.17
24.30	Oxalic acid, allyl undecyl ester	3508937	1.99
24.46	2Hexyl1octanol	1445097	0.82
24.81	1Hexyl2nitrocyclohexane	1492103	0.84
24.91	2(1Methylcyclopentyloxy) tetrahydropyran	2965771	1.68
24.98	1Hexyl2nitrocyclohexane	1289080	0.73
25.40	2Hexyl1octanol	1628030	0.92
25.61	2Hexyl1octanol	1112487	0.63
25.73	1Hexyl2nitrocyclohexane	869336	0.49
25.83	Decane, 3bromo	1415675	0.80
26.06	9Dodecenoic acid, methyl ester, (E)	10132168	5.73
26.20	1Hexyl2nitrocyclohexane	358514	0.20
26.33	1Hexyl2nitrocyclohexane	604817	0.34
26.41	Hexadecanoic acid, 15methyl, methyl ester	11672218	6.61
26.58	2Methyl2chloro3nitroso4cyclohexyloxybutane	1497000	0.85
26.63	Cyclohexanemethyl propanoate	1687922	0.96
26.68	2(1Methylcyclopentyloxy) tetrahydropyran	3612649	2.04
26.90	Oxalic acid, allyl octadecyl ester	4031501	2.28
26.96	2Hexyl1octanol	7972719	4.51
27.07	Decane, 2,5,6trimethyl	885150	0.50
27.20	Oxalic acid, allyl tridecyl ester	5630634	3.19
27.27	Tetradecane, 1iodo	2009711	1.14
27.40	2Hexyl1octanol	2151921	1.22
27.82	2Hexyl1octanol	3458365	1.96

Table 9

Various compounds identified by GC –MS in Dunaliella salina (with Cu stress at 4 ppm)

RT	Compound Name	Area	Area %
4.17	2(1Methylcyclopentyloxy) tetrahydropyran	3715320	3.83
4.21	1Cyclohexylnonene	1177589	1.21
4.26	Cyclohexane, 1(1,5dimethylhexyl) 4(4methylpentyl)	2335885	2.41
4.33	1Hexyl2nitrocyclohexane	1403183	1.45
4.40	Cycloheptene, 5bromo	3025320	3.12
7.31	1,1Dodecanediol, diacetate	2379548	2.45
13.42	Sulfurous acid, butyl decyl ester	936475	0.96
17.41	10Methylnonadecane	991814	1.02
17.93	1,3Dioxolane2heptanenitrile, àmethylëoxo2phenyl	1784052	1.84
21.03	Decane, 3bromo	638395	0.66
21.75	16Heptadecenal	1328764	1.37
22.47	4Propionyloxypiperidine	2257847	2.33
23.31	Dodecanoic acid, 2methyl	3006981	3.10
24.19	Oxalic acid, allyl hexadecyl ester	12126301	12.49
24.30	Oxalic acid, allyl nonyl ester	1391439	1.43
24.37	2,4,6Trimethyl1nonene	1976772	2.04
24.79	Chlorpyrifos	4164169	4.29
24.91	2,2Dimethylpropyl 2,2dimethylpropanethiosulfinate	1670127	1.72
25.83	Dichloroacetic acid, 2,2dimethylpropyl ester	1453917	1.50
25.96	3,4Hexanedione, 2,2,5trimethyl	913878	0.94
26.04	4,8Dioxatricyclo[5.1.0.0(3,5)]octane,	1613583	1.66

	1methyl5(1methylethyl), (1à,3á,5á,7à)		
26.41	Tetradecanoic acid, 10,13dimethyl, methyl ester	3450549	3.56
26.89	1,1Dodecanediol, diacetate	2948898	3.04
26.96	1Hexyl2nitrocyclohexane	3674821	3.79
27.10	Cycloheptene, 5bromo	665373	0.69
27.20	Oxalic acid, cyclobutyl pentadecyl ester	2943594	3.03
27.27	Sulfurous acid, 2propyl undecyl ester	895566	0.92
27.81	2Hexyl1octanol	1183203	1.22
27.90	2Hexyl1octanol	1483536	1.53
28.31	3,4Hexanedione, 2,2,5trimethyl	470655	0.48
28.35	2Hexyl1octanol	540950	0.56
28.68	2Hexyl1octanol	1497008	1.54
28.79	2Hexyl1octanol	1215327	1.25
29.18	2Hexyl1octanol	1287781	1.33
29.67	Decane, 2,5,9trimethyl	1217691	1.25
29.81	2Hexyl1octanol	1051124	1.08
29.97	3,5Pyrazolidinedione, 4(dimethylamino)1,2diphenyl	1514125	1.56
30.03	(2Acetylacetoacetamido) sulphur pentafluoride	1917396	1.98
30.15	2Hexyl1octanol	1527920	1.57
30.28	9Hexacosene	601604	0.62
30.50	2Hexyl1octanol	848547	0.87
30.95	9Hexacosene	1177960	1.21
31.09	2Hexyl1octanol	1603689	1.65
31.32	Sulfurous acid, nonyl 2propyl ester	3141814	3.24
31.42	2Hexyl1octanol	1104002	1.14
31.54	1Hexyl2nitrocyclohexane	2344360	2.42
31.78	1,1Diisobutoxybutane	1939041	2.00
32.34	Decane, 2,5,9trimethyl	919847	0.95
32.44	2Oxecanone, 4hydroxy10methyl, [4R(4R,10R)]	492274	0.51
32.56	Sulfurous acid, butyl nonyl ester	3110701	3.20

CONCLUSION

The present investigation deals with culture and maintenance of various algae for their mechanism of action against various stresses. Further we proceed towards biochemical estimation of these algae and thus we observed that they are good source of proteins and carbohydrates and their content was affected by this heavy metal stress. It was established that significantly less amount of algae has to be consumed to comply with the physiological needs of some relevant microelements, if they are incubated in metal fortified media. The above mentioned results confirm the relevance of development of microelement enriched products based on specifically selected and grown micro alga species. The most efficient ways and conditions of metal bioaccumulation have been established laying the foundations for functional food application in the future.

ACKNOWLEDGMENTS

The authors are thankful to CSIR for providing financial assistance to one of the author Mr. Amrit Daiya and Head, Department of Botany, University of Rajasthan for providing necessary facilities.

CONFLICT OF INTEREST

Conflict of interest declared none.

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